Instructions for use

Roti®-Mark 10-150

Protein-molecular weight marker for SDS-PAGE

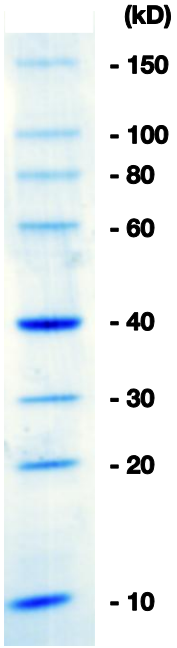


Figure: 4-20 % gradient gel, Tris/SDS

Carl Roth GmbH + Co. KG
Schoemperlenstraße 3-5
76185 Karlsruhe
Postfach 100121
76231 Karlsruhe
Telefon: +49 (0) 721/ 5606-0
Telefax: +49 (0) 721/ 5606-149
E-Mail: info@carlroth.de
Internet: www.carlroth.de

e.d./ c.w. 02/2015

I. Introduction

Roti®-Mark 10-150 is composed of a set of genetically engineered, *in vitro* expressed proteins with defined, regular molecular weights, which stand out for their homogeneous level running properties (see Table 1). This makes it easier to visually estimate the apparent molecular weight of your protein. For easier orientation in the gel, the 40 kD band shows a stronger intensity.
Proteins have been adjusted to equal apparent masses after Coomassie staining. Nearly all bands have a concentration of 50-100 µg/ml per band. The 40 kD protein has been enhanced to 150-200 µg/ml (batch specific).
Roti®-Mark 10-150 has been assembled from a set of **His-tagged, recombinant proteins** that may be detected during Western-Blot analysis using anti-His antibodies.
Proteins are pre-reduced, acylated and dissolved in non-reducing Lämmli buffer with 0.01 % bromophenol blue.

Tab.1:

MW (kD)	logMW
150	5.1761
100	5.0000
80	4.9031
60	4.7782
40	4.6021
30	4.4771
20	4.3010
10	4.0000

In top V you will find a simple procedure for determining the apparent molecular weight of a protein in SDS-PAGE by comparing it to the running distance of marker proteins.

II. Storage

- **The marker will not be shipped cooled or with dry ice. This does not affect usability.**
- Please store Roti®-Mark 10-150 at -20 °C. The marker can be stored at 4 °C for a short period (a few days). To avoid frequent freezing and thawing, aliquots should be frozen.
- If necessary Roti®-Mark 10-150 PLUS can be heated slighty before use to resolubilise precipitated SDS.
- **The marker should not be heated to more than 65 °C nor stored for a longer period at temperatures above freezing point!**

III. Gel loading

- Recommended loading amount for mini gels (10 %; 0.75 mm thick):
  Coomassie-staining: approx. 5 µl
  Silver-staining: approx. 1 µl
- **Please note:** Loading amount required varies depending on gel thickness, C/T ratio, the staining used and width of comb tooth.
- The intensity of Coomassie staining can turn out very differently depending on the protocol used. Two methods which guarantee efficient staining can be found in top VI.

IV. Trouble Shooting

Marker bands cannot/can only be seen very weakly.

- Please ensure the correct loading amount. The recommended quantity is valid for mini gels with a thickness of 0.75 mm. If thicker or larger gels are used, the loading amount must be increased.
- Improve staining: The loading quantity of Roti®-Mark was optimised to obtain particularly sharp bands and optimal running behaviour. Do not try to compensate weak staining by increasing the protein load. This will result in a change of the running

behaviour of the proteins (of your sample as well as of the marker) and in indistinct and thick bands.

- Few weak marker bands: Under certain conditions marker proteins may agglutinate. Resolubilise marker aliquots by incubating for 5 min at 65 °C. Mix carefully.

Marker bands are fuzzy.

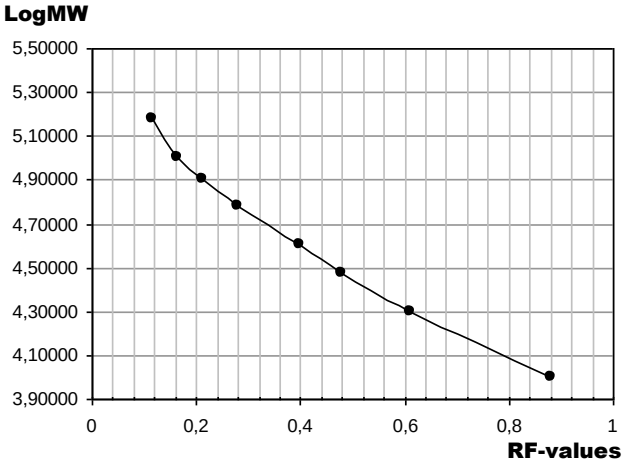
- Avoid overloading the gel!
- Please ensure that the marker is not stored at room temperature for a longer period. Place the marker on ice between two gel runs.
- Avoid frequent freezing/thawing of marker.
- Long-term storage should always take place at -20 °C.
- Please take care that the gel contains no air bubbles when casting.
- When casting the gel, please ensure that the acrylamide solution is mixed thoroughly.
- Only use high quality acrylamide solutions (e.g. Rotiphorese® Gel 30 Art.No. 3029 or Gel 40 Art.No. 3030).
- Avoid overheating the gel. Reduce voltage if required.
- Check the composition and pH value of the buffer used.

V. Apparent MW of a protein

1. Determine the RF values of the marker proteins after electrophoresis.
   Start: Border of collecting /separating gel
   Front of run: Bromophenol blue band

$$RF\ value = \frac{\text{Running distance of protein}}{\text{Overall running distance}}$$

2. Plot log MW (from Table 1) in a graph against the RF values of the marker (example see graph).



3. Calculate the RF value of your protein. Determine the corresponding logMW by using your graph. Calculate the molecular weight in kD according to Table 1.

TIP:
Roti®Mark 10 -150 is composed of genetically engineered multimeres of a protein and exhibits level running properties.
In contrast to this, irregular running properties appear with marker compounds of varying proteins (e.g. myosin, β-galactosidase, BSA, carbonic anhydrase, etc.).
Derived molecular weights of unknown proteins can, therefore, vary, depending on the marker used. Conformation of the values detected with Roti®Mark 10-150 to the traditional marker can be achieved with following formula:

$$MW_{trad} = MW_{detected} - 1.4715 \times \ln(MW_{detected}) + 6.619$$

VI. Coomassie-staining

With Roti®-Blue (Art. No. A152):

- Incubate gel 2 to 12 h with Roti®-Blue as per instructions.
- Decoloring is not necessary.

With Brilliant Blue G250 (Art. No. 9598):

- Incubate gel for 30-60 min in fixative under gentle shaking
- Incubate gel for 20-40 min in staining solution under gentle shaking
- Incubate gel for 30 sec in fixative under gentle shaking
- Incubate gel in decoloring solution under gentle shaking until background staining has been removed and proteins are clearly visible.
- Fixative:
  40 % ethanol, 10 % acetic acid
- Staining solution:
  Mix 50 ml solution I and 50 ml solution II directly before use
  Solution I: 0.2 % Brilliant Blue G250, 90 % ethanol
  Solution II: 20 % acetic acid
- Decoloring solution:
  20 % ethanol, 10 % acetic acid

VII. Recommended Reagents

- Brilliant Blue G250, Art. No. 9598
- Ethanol, p.a., Art. No. 9065
- Acetic acid, p.a., Art. No. 3738
- Roti®-Blue, Art. No. A152

⚠ Warning H319 P305+P351+P338-P337+P313

Roti®-Mark 10-150	
T850.2	**100 µl**
T850.1	**500 µl**